

The University of Chicago

RELATION OF SOIL
TEMPERATURE AND NUTRITION TO
THE RESISTANCE OF TOBACCO
TO *THIELAVIA BASICOLA*

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1937

By

FRANCES LOUISE JEWETT

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Reprinted from
THE BOTANICAL GAZETTE
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TO THE RESISTANCE OF TOBACCO
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CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 494

FRANCES LOUISE JEWETT

(WITH THIRTY-SEVEN FIGURES)

Introduction

Of the diseases which have been studied from the standpoint of the effect of environment on resistance and susceptibility, black root rot of tobacco caused by the fungus *Thielavia basicola* Zopf has received much attention. Field and greenhouse experiments have shown that soil reaction and soil temperature are the most important external conditions determining the severity of the disease. The field experiments of BRIGGS (7), in which he studied the amount of acidity as measured by the lime requirement method, and the later work of ANDERSON (1), DORAN (12), and others in which they measured the intensity of the acidity by determining the pH of the soil, have shown the importance of soil reaction. ANDERSON reports, however, that the range of pH within which tobacco does not become infected with *Thielavia* may vary slightly with the temperature. JOHNSON and HARTMAN (15) tested the effect of various factors, including soil reaction, and concluded that soil temperature is the most important single controlling factor in determining the severity of black root rot of tobacco. Low temperatures, from 17° to 23° C., are the most favorable for development of the disease, while at 29°-30° C. there is very little infection. At 32° C. there is practically no infection, even in the most susceptible varieties of tobacco.

The reasons for this insusceptibility of susceptible varieties at high temperatures are not clear. CONANT (10) reported a distinct correlation between the resistance of the plant and its ability to form cork in advance of the fungus and beneath lesions. The most resistant varieties formed cork readily at low temperatures (20° C.);



the very susceptible varieties formed no cork at low temperatures, but formed it abundantly at the high (30° C.). CONANT states that resistance to *Thielavia* can be accurately measured by the rapidity with which the plant can form cork beneath a lesion.

The first purpose of the present study was to determine the effect of changes in nitrogen nutrition, in conjunction with changes in soil temperature, on the relative resistance of five varieties of tobacco to *Thielavia basicola*. The second purpose was to examine microscopically the roots of these varieties to see the effects of these changes in nutrition and temperature on cork formation. It was also hoped that it could be determined whether a corky layer such as described by CONANT is laid down in advance of or subsequent to fungal invasion, after the initial infection.

Materials and methods

The varieties of tobacco used were: Ordinary White Burley, susceptible; Resistant White Burley, resistant; Connecticut Havana 38, resistant; Havana 142, very resistant; and Xanthia, extremely resistant.

Two cultures of *Thielavia* from the University of Wisconsin, an Italian strain and a Wisconsin strain, proved ineffective in producing infection in tobacco under the conditions of the experiment. New cultures were obtained by isolation from plants grown in infected soil brought from the experimental farm of the University of Wisconsin at Madison. These cultures were used successfully to obtain infection.

The experiments were carried out in the greenhouses of the University of Chicago, using the soil-nutrient temperature tank as described by LINK (19). The plants were grown from seed in sterile soil; when they had reached the six or seven leaf stage, two plants were transferred to each pot of the temperature tank, in which sterile quartz sand was used instead of soil. The temperatures used in all but one series were 28° – 30° C. for one set of tanks and 18° – 20° C. for the other. In one series, grown during extremely hot summer weather, 24° – 26° C. was the lowest soil temperature attainable in the tank.

For each temperature, half of the tanks were given nutrient solu-

tion containing nitrate nitrogen, and the other half, solution lacking the nitrogen. The composition of the nutrient solution given in partial volume molecular concentration is as follows:

SOLUTION	$\text{Ca}(\text{NO}_3)_2$	KH_2PO_4	MgSO_4	CaCl_2
Plus nitrate.....	0.0090	0.0045	0.0045	
Minus nitrate.....		0.0045	0.0045	0.0090

The pH of the solutions made with distilled water is approximately 5.7 and no adjustment was made. Five hundred cc. of the solution was given to each pot of +N plants three times a week, while the -N plants received 500 cc. twice a week.

Five series of plants were grown. In series I and II the plants were supplied with nutrient solution a month before inoculating with pure cultures of *Thielavia*. By this time the external characteristics of the +N and -N plants were evident, and a nitrate test of the -N plants showed an absence of nitrate nitrogen in the tissues. In these series the pH of the sand was kept between pH 5.6 and 5.9 by watering with acidified tap water. Within this range is the zone in which environmental factors other than pH are limiting, according to ANDERSON and others (1). Plants of series III-V were given nutrient solutions and inoculated from two to three days after placing in the tanks. These series were watered with non-acidified water at regular intervals.

The inoculum for series I-III and V was prepared by growing the fungus in Erlenmeyer flasks on potato dextrose agar. After five to twelve days' incubation, when examination showed an abundance of endoconidia, suspensions of the spores were made by pouring sterile tap water into the flasks, shaking vigorously, and scraping the mat with a sterile inoculating needle. Inoculation was accomplished by clearing away the sand around the plant and pouring some of the suspension directly on the roots. The rest of the suspension was thoroughly mixed with the surrounding sand. In the case of series IV a small amount of infected soil from the field was used as inoculum. This was thoroughly mixed with the sand and was very effective in producing infection.

A set of plants of each temperature and plane of nutrition was kept uninoculated as controls. During the growing period the roots

of the controls of series II and V were wounded by pricking with a fine needle, in order to examine the type of wound reaction shown under the different conditions in the absence of the fungus. In the case of the $-N$ plants, the roots are so small that the wound was made at the base of the stem, or crown, which is below the surface of the soil. Since this region is also attacked by the fungus, it is the region studied in most of the $-N$ plants.

At the end of six weeks the plants were removed from the tanks and measured for total height and relative growth of the roots and tops. The roots were carefully washed and examined microscopically for evidences of infection with *Thielavia*, the chief evidence being the presence of the characteristic chlamydospores.

Material from the control and infected plants was killed and fixed in formal acetic-alcohol, imbedded in paraffin, sectioned on the rotary microtome at $10-12\ \mu$, and stained with Flemming's triple stain.

Results

GREENHOUSE EXPERIMENTS

Table 1 summarizes the results obtained in the tank experiments in the greenhouse. Since no record, except failure to obtain infection, was kept of series III, this series was not included in the table. This summary indicates that between the $+N$ and $-N$ plants there is no difference in resistance and susceptibility which is either sufficiently striking or sufficiently consistent to be evident from a limited number of small scale experiments.

Between the high temperature and the low there is a difference both in the number of varieties affected and in the severity of the infection. At the low temperature the number of varieties showing infection is greater, since at this temperature the more resistant varieties immune at high temperatures become infected. The infection is also more severe at the lower temperature, as can be seen from the greater degree of decay of the roots and the greater stunting of the plant as a whole.

Although the number of varieties affected increases with a decrease in temperature, the order of severity of infection parallels the order in which the five varieties used are rated as to suscepti-

bility. This is true for all temperatures and for both +N and -N plants. The combined effect of temperature and the genetic constitution of the variety is seen in series I, in which the fungus culture was very weakly aggressive. The only undoubted case of infection occurred in the most susceptible variety at the low temperature.

The results of these experiments are in agreement with the general finding of others, that of the environmental factors tested, soil temperature is the most important one influencing the expression of

TABLE 1

COMPARATIVE INFECTIONS OF FIVE VARIETIES OF TOBACCO UNDER +N AND -N CONDITIONS AT DIFFERENT TEMPERATURES (IN DEGREES C.)

VARIETY	SERIES I				SERIES II				SERIES IV				SERIES V			
	+N		-N		+N		-N		+N		-N		+N		-N	
	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18- 20	28- 30	18-20	28- 30	18-20	28- 30
Ordinary White Burley.....	+	-	+	-	-	-	-	-	+	+	+	-	++++	++	++++	++
Resistant White Burley.....	-	-	-	-	-	-	-	-	+	+	+	-	++	++	++	++
Havana 38.....	-	-	-	-	-	-	-	-	+	±	+	-	++	++	++	++
Havana 142.....	-	-	-	-	-	-	-	-	±	-	±	-	++	+	++	++
Xanthia.....	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	±

- = no infection; ± = slight infection; +, ++ = fairly heavy to heavy infection; +++ = severe infection.

resistance and susceptibility of varieties of tobacco to *Thielavia*. The results also show the importance of the breeding of resistant strains as a practical method of control, since even at the low temperatures most favorable for the disease, resistant varieties are not so severely affected as susceptible varieties, although they may not be completely immune.

MICROSCOPIC EXAMINATION OF ROOTS

UNWOUNDED CONTROLS.—At both the high temperature (28°-30° C.) and the low (18°-20° C.), a somewhat more regular and compact periderm was found in the +N control plants than in the -N ones. At both temperatures +N susceptible Ordinary White Burley

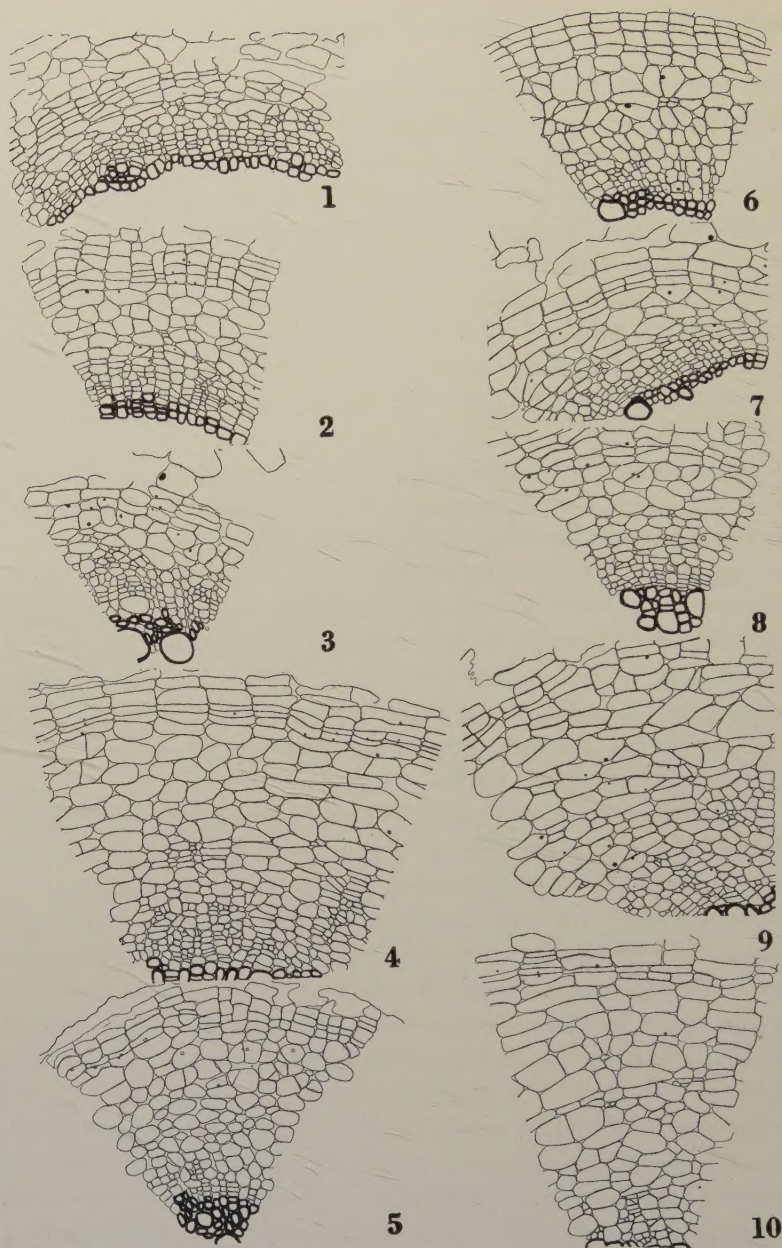
showed as well developed periderm as very resistant Xanthia. In all cases, however, the White Burley was much more severely infected than Xanthia, which remained almost entirely free of infection. The same was true of Resistant White Burley, which is more susceptible than Xanthia. At both temperatures and planes of nutrition the most extensive and continuous periderm was found in the roots of Havana 142, a very resistant variety although not so resistant as Xanthia.

At both temperatures very resistant Xanthia showed more regular periderm formation in the $-N$ plants than did susceptible White Burley.

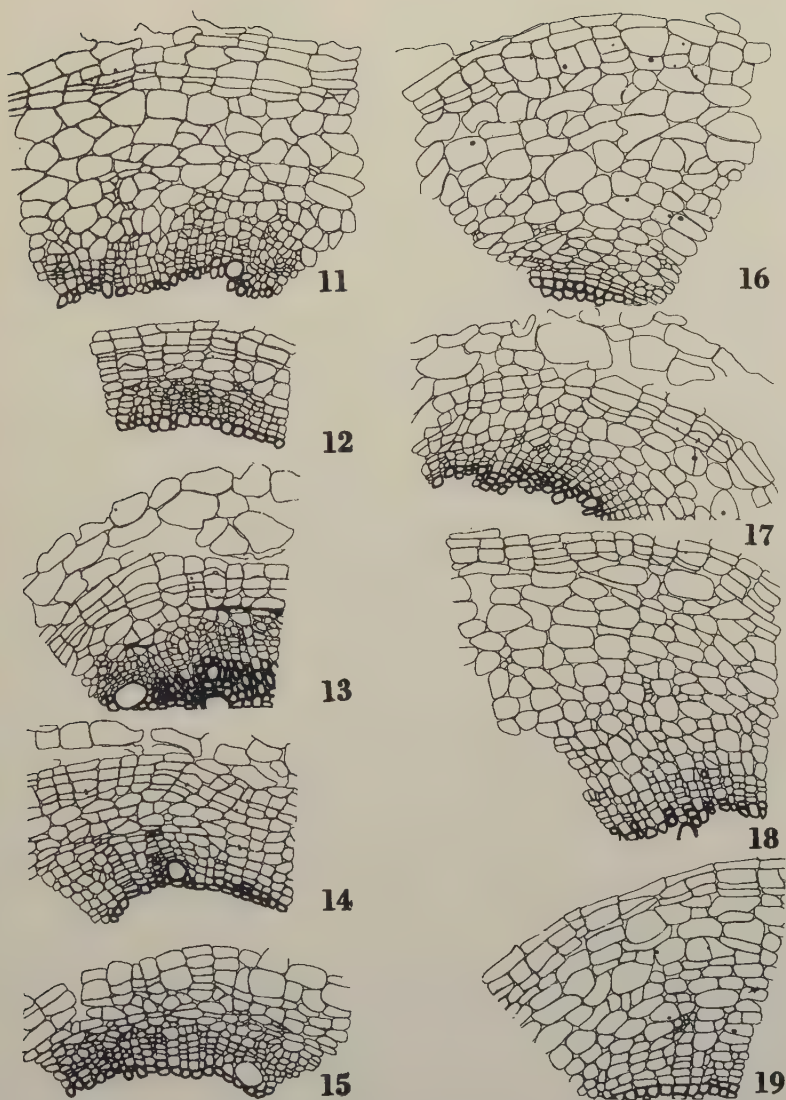
Havana 38, which is intermediate as to resistance, is the only one which showed more regular periderm formation at higher temperatures than at low. This was true for both $+N$ and $-N$ plants. Comparing Havana 38 with Xanthia, it was found that at the higher temperature $+N$ Havana 38 showed slightly better developed periderm than $+N$ Xanthia, while $-N$ Havana 38 had a periderm which was at least as well developed as that of Xanthia. At the lower temperature Xanthia formed a more extensive periderm in both $+N$ and $-N$ plants. At both temperatures Havana 38 was much more severely affected than Xanthia.

Figures 1-19 show portions of the roots of the different varieties studied, giving in each case the region of the best developed periderm noted. The development of a protective layer was by no means uniform, either in extent around the root or in depth and compactness of the periderm cells. Havana 142 showed the most regular development. The thickness of the walls of these cells and their reaction to stains indicated that they were not always very heavily suberized. Their efficiency as a factor in resistance is doubtful.

The base of the stem which is underground, and which may become as heavily infected as the roots, showed no regular protective periderm layer. The epidermal cells were rather heavily suberized, as were the subepidermal cells in some cases. In certain regions a subepidermal periderm had developed, although in many stems no very extensive periderm was formed at any point, nor was the periderm uniform around the whole stem. One side of the stem often



FIGS. 1-10.—Portions of cross sections of roots of control plants grown at 18° - 20° C., showing comparative peripheral periderm development. Figs. 1-5, +N: 1, Xanthia; 2, Havana 142; 3, Havana 38; 4, Resistant White Burley; 5, Ordinary White Burley. Figs. 6-10, -N: 6, Xanthia; 7, Havana 142; 8, Havana 38; 9, Resistant White Burley; 10, Ordinary White Burley.



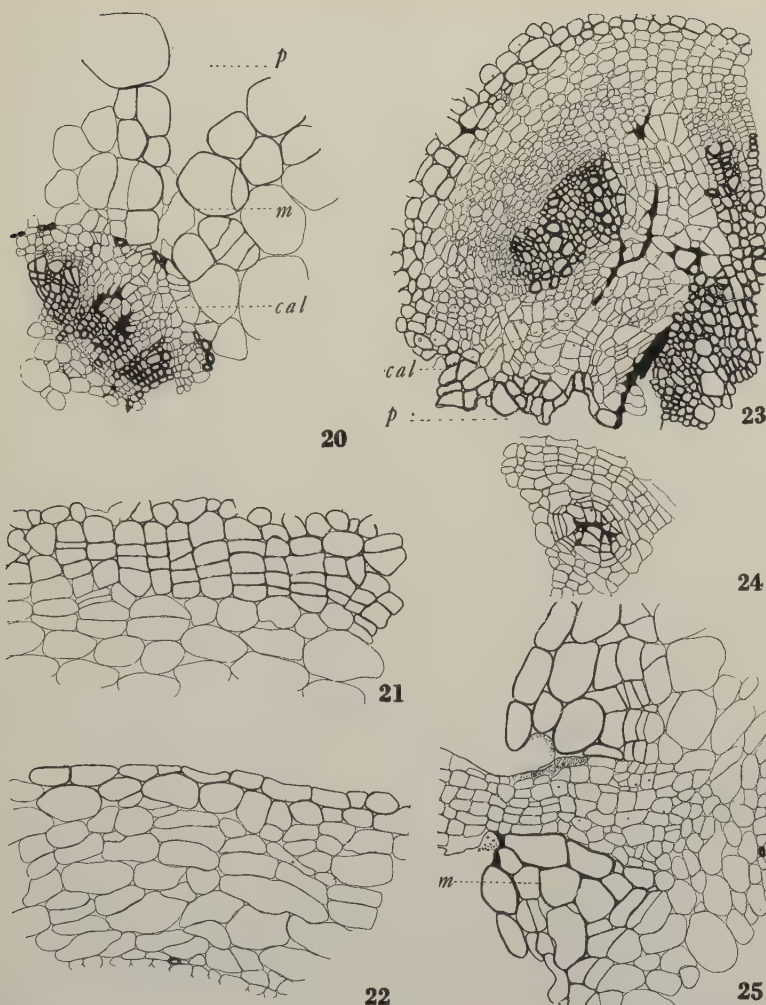
FIGS. 11-19.—Portions of cross sections of roots of control plants grown at 28°-30° C., showing comparative peripheral periderm development. Figs. 11-15, +N: 11, Xanthia; 12, Havana 142; 13, Havana 38; 14, Resistant White Burley; 15, Ordinary White Burley. Figs. 16-19, -N: 16, Xanthia; 17, Havana 38; 18, Resistant White Burley; 19, Ordinary White Burley.

showed a periderm of several cells in thickness, while the other side had only the suberized epidermal cells as protection (figs. 21, 22). So far as could be seen, the structure of the stem with respect to periderm formation was unchanged in relation to differences in temperature and nutrition. No marked differences were observed in the several varieties of tobacco used.

PROTECTION AT BASE OF BRANCH ROOTS.—Examination of the bases of branch roots emerging from both stems and roots showed no consistent formation of periderm as a protective layer in the areas exposed by the breaking through of the emerging root. In the case of secondary roots there was only the slightest evidence of the continuous peripheral cork layer described by CONANT (10). There were very definite reactions in the cortex of the stem in response to the injury made by the emerging adventitious root (fig. 25). The staining reaction indicated that the composition of the cells of the walls of the stem adjacent to the emerging root had changed to a substance staining like lignin or suberin. The walls of such cells also appeared somewhat thickened. Near the periphery of the stem there was some cell enlargement accompanying the change in nature of the cell wall. Very often cell divisions resulted in the formation of a weak periderm-like layer delimiting the wound made by the root emergence. Gum deposits were seen in the small pockets made by the destruction of the cells of the stem. There was apparently no correlation between the intensity of the reaction and the relative resistance of any variety examined. The reactions described were entirely comparable to the responses to other wounds in the cortex, a condition noted by BOYLE (5) in relation to emerging secondary roots in flax.

Examination of cross sections of control roots of as nearly comparable stages of development as possible showed that the amount of normal periderm formed by the five varieties of tobacco under the different conditions of the experiment could not be clearly correlated with the order of the rating of these varieties as to their susceptibility and non-susceptibility to *Thielavia*.

REACTIONS TO INJURY.—The most common response to injury was the change in constitution of cell walls in mechanically injured and infected areas. This change is evident from the reaction of the



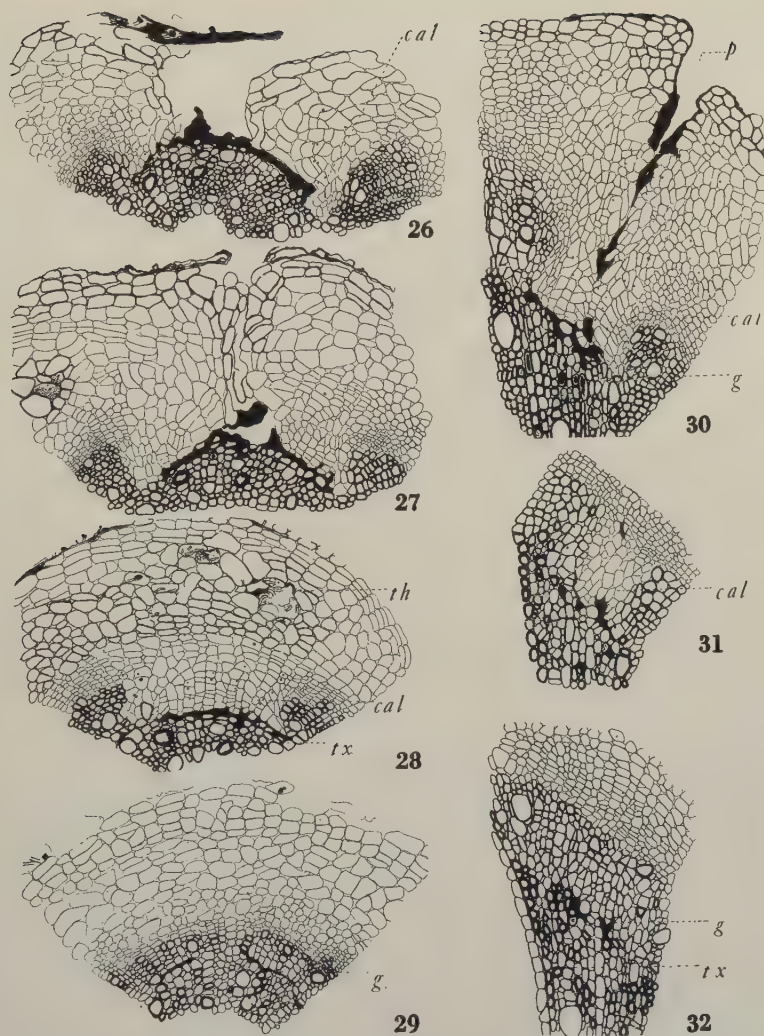
FIGS. 20-25.—Fig. 20, needle wound and reactions in stem, showing metacutinization of cortical cell walls, wound gum, cell divisions in cortex, and callus formation (*p*, path of needle; *m*, metacutinization; *cal*, callus); Havana 142, 18°–20° C., –N; control. Figs. 21, 22, portions of periphery of same cross section of stem base, fig. 21 showing subepidermal periderm on one side and fig. 22 lack of periderm on other side of stem; Havana 142, 18°–20° C., +N; control. Fig. 23, isolation of portions of xylem by callus development stimulated by injury with needle; cells bordering path of needle show metacutinization of walls, indicated by heavier lines; Ordinary White Burley, 18°–20° C., +N; control. Fig. 24, small wound in cortical region of root, showing deposit of gum surrounded by cells reacted by dividing; Ordinary White Burley, 18°–20° C., +N; control. Fig. 25, wound reactions around emerging adventitious root and showing evidence of cell divisions and wound gum formation; Havana 38, 24°–26° C., –N; control.

walls to stains. Cells whose walls stained orange in healthy roots, indicating cellulose with Flemming's triple stain, change to the red of lignified or suberized walls in injured regions. This change in constitution is comparable to the metacutinization of cell walls in injured areas described by KÜSTER (18). In control plants, cells bordering wounds of even very small extent show this reaction. In infected plants this response is found in cells adjacent to those containing the fungus as well as in the infected ones themselves.

Metacutinization is often accompanied by thickening of the wall of infected cells or of cells nearest the region destroyed by the fungus or by mechanical injury. Marked thickening of xylem elements is a characteristic response to injury (figs. 26-28, 32).

In addition to these changes in cell walls, deposits of a dark staining substance, apparently wound gum, are found in injured areas. Small wounds in the cortex result in the formation of pockets of gum surrounded by cells which have reacted by changes in the cell wall and by cell divisions (fig. 24). Similar pockets are found in injuries at the periphery of the root. Heavy gum deposits are seen in severely infected regions and in deep mechanical wounds. These deposits border the area of destroyed cells of the cortex and phloem, and are found on the face of the xylem where the cambium and some of the xylem elements have been destroyed (figs. 26, 27, 30). Gum in and between the xylem elements is one of the first wound reactions visible at a distance above or below the area actually destroyed (figs. 29, 32). In less severe mechanical injury and in infection this substance is seen as a coarsely granular material. In diseased roots it is often seen in the cells containing the fungus, where it appears as a granular coating on the hyphae (fig. 36).

Stimulation to cell division is a further reaction to injury. More or less regular divisions occur in the affected cells around some wounds (figs. 24, 33), but for the most part the divisions are irregular and do not result in the formation of a layer anatomically comparable to a compactly organized periderm. In deep lesions of both mechanical and fungal origin living cells bordering the destroyed areas are stimulated to callus formation (figs. 26-28, 30, 31). Involved in this formation are the cells of the cambium undestroyed at the edges of the wound and the parenchyma cells of the other



FIGS. 26-32.—Comparison of fungus injury with mechanically induced wound: Figs. 26-29, injury caused by *Thielavia basicola*; Havana 142, 18°-20° C., -N; fig. 26, region of greatest tissue destruction; wound gum on face of xylem and callus developing at edges (*cal*, callus). Chlamydospores of *Thielavia* in debris at periphery of root; fig. 27, region just beyond that shown in fig. 26; fig. 28, beyond fig. 27, approaching edge of region which had been destroyed (*th*, hyphae of *Thielavia*); metacutinization of cortical cell walls indicated by heavy lines; wound response in xylem seen in thickening of some xylem elements (*tx*); fig. 29, beyond region of tissue destruction; evidence of injury seen in wound gum (*g*) in xylem. Figs. 30-32, wound mechanically induced by pricking; Ordinary White Burley, 28°-30° C., +N; fig. 30, just above center of path of needle (*p*); fig. 31, region beyond that of fig. 30, approaching limits of the injury, but responses seen in callus and wound gum formation and thickening of xylem elements; fig. 32, beyond region of cell destruction; evidences of wound reaction in xylem in gum deposits and thickened xylem elements.

tissues bordering the injured region. Stimulation of the xylem parenchyma to division often results in the irregular development of the xylem due to the formation of areas of callus tissue isolating one part from the rest (fig. 23). This irregular development was seen in both types of injury, and was also mentioned by CONANT as one of the reactions to the invasion of *Thielavia*.

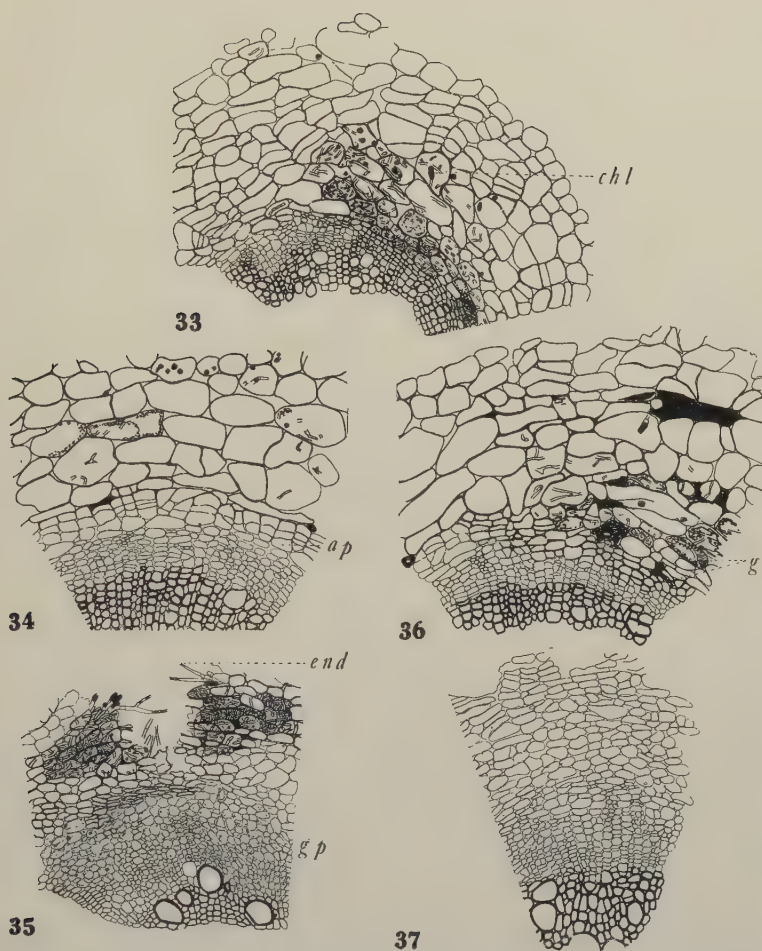
The callus tissue effectively heals the wound. This type of reaction to local injury was found in stems as well as in roots in the five varieties studied under the different conditions of the experiment (fig. 20). No differences were noted in amount or type of tissue formed which might be correlated with resistance and susceptibility.

One reaction seen in infected roots was not seen in those mechanically injured. Beneath heavily infected areas, the protoplasm was more granular and stained much more deeply than the protoplasm in similar cells in healthy areas of the same root (figs. 35, 37). This response to the fungus was found in these roots only when infection was rather severe. TISDALE (26) described a similar appearance in the cells of infected flax roots, and suggested that the change might be due to a chemical reaction brought about by the presence of the fungus.

These microscopic examinations of roots and crowns of tobacco plants infected with *Thielavia basicola* under the conditions of this experiment failed to corroborate the findings of CONANT with respect to periderm formation around lesions resulting from the presence of the fungus. A comparison of injuries made by *Thielavia* with those induced mechanically showed that the reactions to wounding were of the same type regardless of the cause of injury, and that other reactions were much more frequent than periderm formation.

Discussion

Although series I-III were of no great value in providing answers to the main questions under investigation, they were of interest since they apparently showed that *Thielavia basicola* may lose its virulence after long culturing on artificial media. This is not in accordance with the experience of JOHNSON and HARTMAN (15), who found no evidence in the literature or in their experiments of variation in virulence due to the age of the culture or to differences in



FIGS. 33-37.—Fig. 33, infected stem base showing cell divisions between fungus and periphery of stem; infected cells and those adjacent show metacutinization of walls (*chl*, chlamydospores of *Thielavia*); Havana 38, 28°-30° C., -N. Fig. 34, evidences of activity in pericycle adjacent to infected cells of cortex; walls of cortical cells and outer cells of pericycle, metacutinized (*ap*, activity in pericycle); Ordinary White Burley, 18°-20° C., +N. Fig. 35, infected root showing granulation of protoplasm in cells beneath heavily infected area (*end*, endoconidium; *gp*, granular protoplasm). No evidence of periderm formation around fungus lesion; Ordinary White Burley, 28°-30° C., +N. Fig. 36, same stem base shown in fig. 34. No evidence that activity in pericycle has formed barrier of periderm cells in advance of fungus. Walls of infected and adjacent cells metacutinized, and granular gumlike material seen in some cells. Fig. 37, healthy portion of root shown in fig. 35; protoplasm of cells not granular.

strain, and that *Thielavia* is apparently stable in its pathogenicity. PETERS (21) and SATTLER (23) on the other hand both report loss of virulence after long cultivation on artificial media, two to three years according to PETERS. Both also report evidences of different biologic races of *Thielavia*.

The relation of soil temperature to disease has been fully discussed by JONES, JOHNSON, and DICKSON (16) and others. The effects of temperature on the resistance and susceptibility of tobacco to *Thielavia* have been well established by field observations and experiments, although the mechanism by which high temperature increases resistance is not understood.

Work on the relation of nutrition to resistance and susceptibility shows that, owing to the many factors involved in the nutrition of a plant, it is difficult to make generalized statements. FISCHER and GAÜMANN (13) state that in general, high nitrogen nutrition increases susceptibility, while high phosphorus, potassium, and calcium lessen it. They state that it is necessary to have extremes of high or low nutrition with these elements for the changes in resistance to be evident. Furthermore, the effects of these elements are not always the same. In certain diseases, high nitrogen may increase resistance and high phosphorus decrease it.

SCHAFFNIT and VOLK (24), working on the relation of various individual nutrients to resistance of both herbaceous and woody plants, found that plants with a decided lack of nitrogen were only slightly susceptible while those with a decided excess were especially susceptible. This was true wherever susceptibility and resistance were independent of the age of the host. In plants in which age affects resistance, this relationship to nitrogen may be reversed in the early stages of growth, while in the older stages the +N plants are again more susceptible.

LINK and WILCOX (20) report that succulent shoots of Stayman apple trees, such as formed under high nitrogen conditions, are susceptible to *Erwinia amylovora*, while the -N shoots are only slightly disposed toward the organism.

In the case of stem rust of wheat, a high amount of nitrogen is known to increase development of the rust. JOHNSON and JOHNSON (14) studied the nitrogen content of mature and immature parts of

the wheat plant in relation to resistance to the rust. They found that the mature parts had a higher nitrogen content than the immature parts, and at the same time were more resistant than the immature. They concluded that resistance cannot be due to the actual increase in nitrogen content, as believed by GASNER and FRANKE, but that in the older tissues other factors enter in, such as the increased resistance offered by the more mature cells or the development of some substance having an inhibiting effect on the fungus. They believe that resistance to rust cannot be explained on a directly nutritional basis.

Reports on the effects of nutrition on resistance to root fungi are not numerous, and the conclusions are somewhat divergent. SATTLER (23) found that beans were more susceptible to *Thielavia* under conditions in which nitrogen was either completely lacking or greatly in excess.

COOK (11) found that both resistant and susceptible tomato plants showed a high frequency of infection with *Fusarium lycopersici* under $-N$ conditions, although the symptoms of wilt were infrequent in $-N$ plants. Seedlings of a resistant variety were infected under both $+N$ and $-N$ conditions, showing symptoms similar to those of susceptible varieties.

REED and FRÉMONT (22) found that citrus roots growing in soils which had been without fertilizer for seven years offered little resistance to the invasion of mycorrhizal fungi and were unable to digest intracellular mycelium. Roots in soils having had annual cover crops and application of stable manure offered a definite resistance to the fungus.

THOMAS (25) states that trees infected with *Armillaria mellea* develop an antagonism to the fungus only when the plant is in an active healthy state, a condition which implies at least no lack of nitrogen.

The tobacco plants examined in the present study showed no anatomical modifications in response to the presence of *Thielavia* which could be correlated with resistance. No such periderm layers as described by CONANT were found; and while evidences of cell divisions around some infected areas were noted, these were not found consistently enough, either in a given diseased root or under

a given set of conditions, to be considered a factor in resistance. One of these regions showing such activity in the pericycle occurred in susceptible White Burley at the low temperature, the temperature condition under which the plant was the most susceptible (fig. 34). Other parts of the same crown showed that the fungus had not been stopped by a periderm-like barrier, but had penetrated the phloem almost to the xylem (fig. 36). This same irregularity of cell division in response to the presence of the fungus was seen in the other varieties used in this experiment.

Periderm formation has received much attention as a possible factor in determining the ability of a plant both to resist initial invasion and to prevent further penetration after invasion. The work of ARTSCHWAGER and STARRETT (4) on sweet potato and gladiolus shows the importance of the ability of the root to heal rapidly the wounds made in harvesting. Unhealed wounds are portals of entry for pathogenic organisms during storage. Under conditions of high temperature and relative humidity, when periderm is formed most rapidly, rotting in storage is much decreased. According to APPEL (2) the cork layer must be thick, for many fungi are able to penetrate thin cork layers.

As to the effectiveness of a corky layer in preventing continued penetration after initial invasion, there is a variety of findings. BROOKS (8) states that with certain varieties of plants, resistance to some fungus diseases is related to the ability of the host to form cork barriers readily in response to attempted invasion. This cork may apparently completely check the advance of the fungus, so that its harmful effect on the plant as a whole is decreased. In the case of rough bark of aspen, caused by *Macrophoma tumefaciens*, as described by KAUFERT (17), the fungus penetrates the periderm. The host forms a new periderm in response to the wounding; the fungus penetrates this, and the alternate penetration and formation of new layers continue until there is a very thick layer of cork. This slow progress of the fungus restricts the organism to the outer cortex although it may be present there for years.

THOMAS (25) found that the wound made by the invasion of *Armillaria mellea* in resistant hosts is walled off by a periderm, but

he doubts the significance of the cork layer as a factor in resistance, since the fungus easily breaks through such layers.

Much of the literature indicates that where cork formation is observed, it is not considered to be the only factor operative in resistance but functions in conjunction with other factors. APPEL (2) found in black leg of potato that the rapidity with which the potato is able to form cork in response to the wound is related to the ability to resist further invasion. But he also states that the efficiency of the cork layer is aided by conditions that diminish the growth of the bacteria, giving the wound time to heal before the bacteria can penetrate. In the case of fungus diseases such a newly formed cork is not effective, he thinks, as fungi are able to penetrate it.

LINK and WILCOX (20) found that a periderm walled off lesions made by *Erwinia amylovora* in apple shoots. But they also noticed that there was usually a band of unaffected cells between the bacteria and the periderm. The bacteria were evidently stopped first and the periderm was formed later as a regenerative reaction to the wound, rather than in advance as a defense mechanism.

TISDALE's work on flax is often quoted as describing a situation in which the flax wilt fungus is prevented from invading the vessels in resistant varieties by the formation of a corky barrier between the fungus and the vessels. TISDALE in fact pointed out that the formation of a corky barrier was but one of a number of cellular changes, and that a substance was formed by the host cells which was possibly harmful to the fungus. He surmised that failure of the fungus to penetrate the periderm layer may be conditioned only after the fungus has been weakened by some activity on the part of the protoplasm of the host. Thus resistance resulted from a combination of the weakening of the parasite and the formation of a corky barrier.

The failure of BOYLE (5) to find in flax roots a corky layer such as described by TISDALE may be additional evidence that periderm formation is not the primary factor in resistance in flax roots.

BRAMBLE (6) was uncertain of the complete effectiveness of a wound periderm in preventing the spread of *Endothia parasitica* in chestnut. He thought, however, that it might delay or stop the ad-

vance of the fungus under conditions where the fungus grows slowly because of some other inhibiting factor. BRAMBLE concluded that wound periderm formation should at least be considered among the factors contributing to resistance to *Endothia*.

The metacutinization and thickening of the cell walls in infected areas might offer some mechanical resistance to invading fungi. But there was no evidence in the tobacco roots of this experiment that the fungus is restricted by these features, nor was there any correlation between their occurrence and resistance. From the comparable reactions in mechanically injured roots it is concluded that these are the usual wound responses.

Both TISDALE and BOYLE described metacutinization in flax, but from microchemical tests BOYLE concluded that the substance deposited in the cell walls is lignin-like. He found that this metacutinization is of the same type as that found in reactions to mechanical injuries, and he thought that the deposition of lignified material on the cell wall could not be considered a primary factor in resistance. BRAMBLE described similar reactions in chestnut, and concluded that lignification of cells was not of great importance in resistance to further invasion, since these cells also become infected.

BOYLE, however, found that a greater stability of the cortical cell walls was correlated with resistance. This stability was not evident anatomically but was detected by testing the resistance of the walls to hydrolysis with sulphuric acid and determining the amount of non-hydrolyzable materials in the walls.

Callus formation occurring as a result of severe injury is primarily a regenerative rather than a directly defensive reaction. Its occurrence in regions infected severely enough to result in the destruction of areas of cells suggests that the fungus has been weakened or stopped in its progress, giving the cells which have not been destroyed the opportunity to divide and begin regeneration. The newly formed callus is apparently not infected by the fungus remaining near the new tissue. This may be a further indication that the aggressivity of the fungus has been decreased, either because the organism has been weakened by some substance formed in the cells in response to the fungus, as suggested by TISDALE, or because the nutritive conditions are no longer favorable to the fungus. On the

other hand, the lack of infection in the new callus may suggest some chemical characteristic of this tissue as yet not understood. Anatomically callus does not offer a mechanical barrier to invading fungi, because of its thin walled cells.

Since no anatomical modifications were found in the tobacco plants of this experiment which could explain resistance and susceptibility to *Thielavia*, it seems probable that the mechanisms of resistance are chemical. TISDALE'S suggestion of a fungus-weakening substance, the description by THOMAS of the development in roots of an antagonism toward *Armillaria mellea*, the mention by others of the formation of inhibiting substances, and the work on acquired physiological immunity in plants summarized by CHESTER (9), all show the trend of thought in the direction of a chemical basis for resistance. More specifically, the reports of ARNAUDI (3) that he was able to increase the resistance of tobacco plants to *Thielavia* by treating the plants with vaccines is suggestive of an approach to the understanding of the problem of resistance in the case of black root rot of tobacco.

Summary

1. Plus and minus nitrogen tobacco plants, of the five varieties used in this experiment, showed no differences in resistance and susceptibility to *Thielavia basicola* at any given temperature. Changes in nutrition do not change the order of resistance.

2. These experiments confirm the evidence of other investigators that soil temperature is the most important environmental condition determining the expression of resistance and susceptibility of tobacco to the fungus. The breeding of resistant varieties is the most important practical method of control of black root rot.

3. Periderm formation in control plants at different temperatures and under +N and -N conditions could not be correlated with resistance.

4. No evidence was found of the formation of periderm in advance of the fungus or around lesions made by the fungus.

5. Response to the wounds made by the fungus was similar to the reaction to mechanical wounds.

6. Callus formation in roots and stems in response to injury by the fungus appears to be regenerative rather than defensive.

7. Resistance to *Thielavia* under certain environmental conditions is not determined primarily by anatomical modifications in the root or crown of the plant.

The writer expresses grateful appreciation to Dr. G. K. K. LINK and other members of the department of botany of the University of Chicago for their assistance and criticisms during the course of the work, and to Dr. JAMES JOHNSON of the department of horticulture of the University of Wisconsin for his kindness in supplying material for the study.

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